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L1: Entry 34 of 36 File: USPT Sep 16, 1997

DOCUMENT-IDENTIFIER: US 5667782 A
TITLE: Multiple particulate antigen delivery system

Brief Summary Text (12):
The present invention provides VLPs and CLPs which are immunogenic and especially such VLPs and CLPs which are useful in vaccine formulation. More particularly, the present invention provides genetically engineered, multi-component, virus-like particles (VLPs) and virus core-like particles (CLPs) as vaccine delivery systems for multiple immunogens representing viruses, bacteria and bacterial toxins that are responsible for human diseases (e.g. Hepatitis B, HIV, Respiratory Syncytial Virus, Clostridium difficile, Bovine Leukemia Virus, Helicobacter pylori, etc.),

Brief Summary Text (22):
Genes coding for the Helicobacter pylori urease subunits A and B were produced by PCR and cloned in the pAcUW3 vector. Recombinant baculoviruses were produced using this plasmid. S. frugiperda cells infected with the recombinant baculovirus produced both urease subunits A and B.

Drawing Description Text (20):
FIG. 18 shows PAGE of the lysates of S. frugiperda cells infected with recombinant baculovirus expressing the Helicobacter pylori urease A and B subunits;

Drawing Description Text (21):
FIG. 19 shows Western blot of the lysates of S. frugiperda cells infected with recombinant baculovirus expressing the Helicobacter pylori urease A and B subunits;

Detailed Description Text (77):
Expression of Helicobacter pylori urease subunits A and B epitiopes as CLPs was investigated as follows. Helicobacter pylori is a causative agent of peptic ulcer. Production of Helicobacter pylori urease is of interest for the development of a vaccine against this disease (33).

Detailed Description Text (78):
Dual baculovirus expression vector pAcUW3 was used for the expression of both A and B subunits of Helicobacter pylori using single baculovirus (34). Polymerase chain reaction (PCR) was performed as described (30). For construction of the recombinant baculovirus, the plasmid transfer vector containing urease A and B subunits was lipofected with Bsu36.1 cut BacPAK6. DNA, white plaques were selected and purified by two sequential plaque assays (31). Polyacrylamide gel electrophoresis were done as described before (28).

Detailed Description Text (79):

Genes coding for the Helicobacter pylori urease subunits A and B were produced by PCR, using 2.7 kb TaqI clone (W. Thomas) as a template. PCR fragments containing structural genes coding for the urease subunits were cloned in the pAcUW3 vector, urease A subunits in the BgHI site, under the control of the polyhedrin promoter. Orientation of PCR fragments in the pAcUW3 A, B. plasmid was established using HindIII restriction endonulclease.

CLAIMS:

3. The antigen of claim 1, wherein the foreign epitope is from a rabies virus protein, a human hepatitis B virus protein, a human immunodeficiency virus protein, or a Clostridium difficile protein.

L1: Entry 31 of 36 File: USPT Jul 6, 1999

DOCUMENT-IDENTIFIER: US 5919463 A
TITLE: Clostridium difficle toxins as mucosal adjuvants

Brief Summary Text (5):
We have shown that C. difficile toxins, when administered intranasally with an antigen (e.g., Helicobacter pylori urease, ovalbumin (OVA), or keyhole limpet hemocyanin (KLH)) are effective in inducing mucosal immune responses to the antigen. For example, the immune response to H. pylori urease induced upon intranasal administration of urease with a C. difficile toxin is protective against Helicobacter infection. We have also shown that intranasal administration of an antigen with a non-toxic derivative of C. difficile Toxin A, containing the carboxyl-terminal repeats which make up the carbohydrate binding domain, leads to a mucosal immune response. In addition, we have shown that rectal and vaginal immunization routes are effective. Thus, C. difficile toxins, and fragments thereof, are effective mucosal adjuvants which can be used in vaccination methods.

Brief Summary Text (10):
The toxins used in the invention may also be produced as fusion proteins. A fusion protein is a polypeptide containing amino acid sequences corresponding to two or more proteins (or fragments thereof), which are normally separate proteins, linked together by a peptide bond(s). Fusion proteins generally are synthesized by expression of a hybrid gene containing nucleotides encoding each of the individual polypeptides which make up the fusion protein. An example of a fusion protein included in the invention is one which contains a Clostridium (e.g., C. difficile) toxin (e.g., C. difficile Toxin A or B; or a fragment or derivative thereof having adjuvant activity) fused to an antigen, e.g., H. pylori urease. Another type of fusion protein included in the invention consists of a C. difficile toxin fused to a polypeptide (e.g., glutathione S-transferase (GST)) which facilitates purification of the fusion protein. Toxins used in the invention may also be covalently coupled or chemically cross-linked to an antigen, using standard methods.

Brief Summary Text (12):
Any antigen to which a protective and/or therapeutic immune response is desired may be administered with an adjuvant of the invention. Exemplary organisms from which antigens (which may be, e.g., subunit antigens, killed whole cells, or lysates) may be derived include, but are not limited to, Helicobacters (e.g., H. pylori, H. felis, and H. heilmanii), Campylobacters (e.g., C. jejuni), Clostridia (e.g., C. difficile), Corynebacterium diphtheriae, Bordetella pertussis, influenza viruses, parainfluenza viruses, respiratory syncytial virus, Borrelia burgdorferi, Plasmodium, herpes simplex viruses, human immunodeficiency virus, papilloma viruses, Vibrio cholera, Escherichia coli, measles virus, rubella virus, varicella-zoster virus, mumps, rotavirus, shigella, Salmonella typhi, Neisseria gonorrhoeae, Yersinia, Treponema pallidum, hepatitis viruses, and Chlamydia. In addition, vaccines against nonmicrobial pathogens, e.g., vaccines containing killed cancer cells, or cancer cell-specific or enriched antigens, may be administered with the adjuvants of the invention.

Brief Summary Text (14):
The invention also features a composition containing an antigen and a toxin (or toxins) from a Clostridium (e.g., C. difficile, C. novyi, C. sordellii, C. perfringens, C. tetani, and C. botulinum), or a fragment or derivative thereof having adjuvant activity, in a pharmaceutically acceptable vehicle (e.g., water, a saline solution (e.g., phosphate-buffered saline), a bicarbonate solution (e.g., 0.24 M NaHCO.sub.3), or in the form of a suppository, depending on the immunization route selected). Toxins which may be contained in the compositions of the invention are described above and include, e.g., C. difficile Toxin A, C. difficile Toxin B, both C. difficile Toxin A and C. difficile Toxin B, or fragments (e.g., a carboxyl-terminal fragment containing the repeats which make up the carbohydrate binding domain of Toxin A (ARU)) or derivatives thereof having adjuvant activity. The toxins may be recombinant, synthetic, part of a fusion protein (which includes, e.g., an antigen (e.g., Helicobacter pylori urease) or a polypeptide (e.g., GST) which facilitates purification of the fusion protein), covalently conjugated to an antigen, chemically cross-linked to an antigen, or toxoided (i.e., rendered less toxic, see above). Examples of antigens that may be contained with the adjuvants in the compositions of the invention are listed above.

Drawing Description Text (6):
FIG. 3 is a graph showing the urease activity in gastric tissue of mice immunized intranasally with urease (5 .mu.g) in combination with the indicated adjuvants, and subsequently challenged with virulent Helicobacter felis (see above description of FIG. 2). Gastric tissue samples were taken 2 weeks after H. felis challenge.

Detailed Description Text (7):
Fusion proteins containing a Clostridium (e.g., C. difficile) toxin (or a fragment or derivative thereof having adjuvant activity) fused to, e.g., an antigen of a pathogen (e.g., H. pylori urease), are also included in the invention, and may be prepared using standard methods (see, e.g., Ausubel et al., supra). In addition, the toxin adjuvants of the invention may be covalently coupled or cross-linked to antigens (see, e.g., Cryz et al., Vaccine 13:67-71, 1994; Liang et al., J. Immunology 141:1495-1501, 1988; and Czerkinsky et al., Infection and Immunity 57:1072-1077, 1989).

Detailed Description Text (26):
Adjuvant Activity of C. difficile Toxins with H. pylori Urease

Detailed Description Text (27):
The following experiments show that mucosal immune responses to H. pylori urease are enhanced when the antigen is administered intranasally with a low, non-toxic dose of C. difficile Toxin A or C. difficile Toxin B, and further that this immune response is protective against subsequent Helicobacter challenge.

Detailed Description Text (28):
H. pylori urease apoenzyme (5 .mu.g) purified from recombinant E. coli expressing the urease structural subunits (Lee et al., supra) was administered intranasally to female Swiss Webster mice (5 mice/adjuvant) once per week for four consecutive weeks in combination with C. difficile Toxin A (0.2 .mu.g), C. difficile Toxin B (1 .mu.g), C. difficile Toxoid culture filtrate (containing 15 .mu.g each Toxin A and Toxin B inactivated with 1% formalin), CT (5 .mu.g), the B subunit of CT (CTB; 5 .mu.g)+CT (10 ng), or without adjuvant. The adjuvancy of the toxins was determined by: (1) measuring the induction of urease-specific mucosal IgA, and (2) observing the induction of protective immunity.

Detailed Description Text (36):
FIG. 3 shows the results of the gastric urease assay, and the protection results are summarized in Table 3. Urease alone and urease administered with C. difficile toxoid were ineffective in inducing protective immunity in challenged animals. Urease administered with CT or CT+CTB completely protected 4/4 and 5/5 animals,

respectively, while urease administered with Toxin A completely protected 3/5 animals and partially protected 5/5. Wilcoxon ranked sums analysis showed that the magnitude of infection, as determined by measuring the OD.sub.550, was significantly lower in the animals administered urease+Toxin A, compared with those administered urease alone (p=0.0122). Animals immunized with urease+Toxin B also had lower grade infections than those immunized with urease alone, and 4/5 animals immunized with urease+Toxin B were partially protected. These data show that C. difficile Toxin A and Toxin B, when administered intranasally with H. pylori urease, induce protective immunity to H. felis challenge.

Detailed Description Text (52):
After the final immunization, samples of serum and mucosal secretions were taken for measurement of anti-ovalbumin antibody levels by ELISA, as is described above. Serum anti-ovalbumin IgG immune responses were enhanced when the antigen was administered with Toxin A by the rectal or vaginal route, but not when the antigen was administered alone (FIG. 8). The anti-ovalbumin IgA antibody response was greatly elevated in feces, and slightly enhanced in serum, saliva, and vaginal secretions, when Toxin A was used as an adjuvant (FIG. 8). All immune responses measured after rectal immunization of ovalbumin+adjuvant were statistically greater than those measured after rectal immunization of ovalbumin alone (Table 6). A similar trend is apparent with vaginal immunizations, but larger numbers of mice are required to demonstrate statistical significance. Interestingly, the vaginal anti-ovalbumin IgG response was elevated when ovalbumin was administered rectally or vaginally with adjuvants, as compared to when the antigen was administered alone. These data show that Toxin A increases specific antibody levels in serum and mucosal secretions when administered to the rectal or vaginal mucosa. As further support for the efficacy of the rectal route, using challenge and assay methods similar to those described above, mice rectally immunized with Helicobacter urease and Toxin A were shown to be protected against Helicobacter challenge.

Other Reference Publication (2):
Davin et al., H. pylori Urease Elicits Protection Against H. felis Infection in Mice, Gastroenterology 104:A1035 (Apr. 1993).

Other Reference Publication (3):
Pallen and Clayton, Vaccination Against Helicobacter pylori Urease, The Lancet 336:186-187, 1990.

Other Reference Publication (42):
Lee et al., Oral Immunization with Recombinant Helicobacter pylori Urease Induces Secretory IgA Antibodies and Protects Mice from Challenge with Helicobacter felis, J. Infectious Disease 172:161-172, 1995.

CLAIMS:

1. A method of inducing an immune response to an antigen in a mammal that is protective or therapeutic against infection, said method comprising administering to said mammal an adjuvant consisting of (a) an enterotoxin of a bacterium of the genus Clostridium, or (b) a polypeptide comprising the carbohydrate-binding domain of a clostridial enterotoxin and an antigen wherein said antigen is distinct from (a) or (b).

8. The method of claim 1, wherein said bacterium is selected from the group consisting of Clostridium difficile, Clostridium sordellii, and Clostridium novyi.

9. The method of claim 1, wherein said bacterium is Clostridium difficile.

10. The method of claim 1, wherein said adjuvant is Clostridium difficile Toxin A.

11. The method of claim 1, wherein said adjuvant is Clostridium difficile Toxin B.

12. The method of claim 1, wherein said antigen is administered with Clostridium difficile Toxin A or a polypeptide comprising the carbohydrate-binding domain of a clostridial Toxin A, and Clostridium difficile Toxin B.

20. The method of claim 19, wherein said polypeptide antigen comprises Helicobacter pylori urease.

21. A method of inducing an immune response to Helicobacter pylori urease in a mammal that is protective or therapeutic against Helicobacter infection, said method comprising administering to a mucosal surface of said mammal Helicobacter pylori urease and an adjuvant selected from (a) an enterotoxin of a bacterium of the genus Clostridium, and (b) a polypeptide comprising the carbohydrate-binding domain of a clostridial enterotoxin.

WEST Search History

DATE: Tuesday, August 27, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	clostridium.clm. and (helicobacter or pylori or hpylori)	36	L1

END OF SEARCH HISTORY

WEST Search History

DATE: Tuesday, August 27, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	parenteral same (oral or mucosal or nasal or vaginal)	32081	L1
L2	L1 same pylori	8	L2
L3	viral near2 mennin$	2	L3

END OF SEARCH HISTORY

Prospects for the development of a vaccine against Helicobacter **pylori.**

Telford J L ; Ghiara P

IRIS, Chiron-Biocine Institute for Immunobiological Research, Siena, Italy.

Drugs (NEW ZEALAND) Dec **1996** , 52 (6) p799-804, ISSN 0012-6667

Journal Code: 7600076

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Over 50% of the world population is chronically infected by the gastric pathogen, **Helicobacter** pylori, which is responsible for most peptic ulcer disease and is closely associated with adenocarcinoma of the stomach. Current therapies for peptic ulcer disease include antibiotic eradication of H. pylori infection. While effective, the high cost, difficulty of patient compliance with the treatment regimens, and risks of selection for resistant strains make these therapies impractical on a large scale. Studies of the pathogenesis of H. pylori have led to the identification of bacterial antigens as candidates for inclusion in novel vaccines against this disease. Both prophylactic and therapeutic vaccination have been demonstrated in animal models of **Helicobacter** infection. Preclinical evaluations of several antigens are at present under way and trials of vaccination in humans are planned. (36 Refs.)

Tags: Human

Descriptors: Bacterial Vaccines--therapeutic use--TU; * **Helicobacter** Infections--therapy--TH; * **Helicobacter** pylori--immunology--IM; *Peptic Ulcer--microbiology--MI; Disease Models, Animal; **Helicobacter** Infections --prevention and control--PC; **Helicobacter** pylori--pathogenicity--PY; Peptic Ulcer--therapy--TH

CAS Registry No.: 0 (Bacterial Vaccines)

Record Date Created: 19970313